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# Secondary Metabolites of Ethanol Extracts of *Pinus sylvestris* Cones from Eastern Anatolia and Their Antioxidant, Cholinesterase and α-Glucosidase Activities

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**Abstract:** Scots pine (*Pinus sylvestris*) is the most widely distributed tree species among pine species. Antiradical, antioxidant properties and inhibition properties on butyrylcholinesterase **(**BChE), acetylcholinesterase (AChE) and α-glycosidase activities of ethanol extracts of *P. sylvestris* were reported from Sarikamis (Kars), Gumushane and Erzurum provinces of Turkey. The cones of *P. Sylvestris* from Gumushane showed the highest $IC_{50}$ values in both $DPPH^{\cdot}$ (14.75 µg/mL) and $ABTS^{\cdot+}$ (12.56 µg/mL) radical scavenging activities. An LC-HRMS method developed and the secondary metabolite composition of extracts were identified. The major compounds are determined as (+)-*trans* taxifolin, quercitrin, fumaric acid, (-) epicatechin, and nepetin-7-O-glucoside, apigenin-7-O-glucoside in *P. sylvestris* cones.

**Keywords:** *Pinus sylvestris*, acetylcholinesterase; butyrylcholinesterase; antioxidant activity; α-glucosidase; LC-HRMS. 

## 1. Introduction

Oxidation is a chemical reaction that separates electrons from an atom or molecule. If an atom loses its electron, it becomes oxidized. It represents an important part of our metabolism and aerobic life. Because of oxygen is used for energy producing in the form of ATP as the final electron acceptor in the electron transport system. A glitch in the transmission of these electrons causes serious problems [1]. Free radicals are extremely unsteady molecules. Molecules, atoms, or groups of atoms that can independently sustain their existence, containing one or more unpaired electrons, are called free radicals [2]. They react rapidly with other molecules to share these electrons. Free radicals are molecules that attack somatic cells and the immune system. Free radicals form the basis for electron transfer, energy production and other metabolic functions [3]. However, radicals become reactive because of the reduction of an electron in molecules entering the reaction, and this reaction causes uncontrolled behavior while continuing to chain, causing damage to the cell [4].

Active species derived from oxygen, including free radicals, play a role in tissue damage following ischemia and reperfusion of the heart and brain. Reactive oxygen species (ROS) occurs continuously during normal physiological events [1]. There is a continuous radical production due to various physical and chemical events in the environment. Too many and various radicals are produced in cellular conditions [3].

Antioxidant defense systems have been developed under normal physiological conditions to prevent the formation and release of free oxygen radicals and to remove them [4]. One of the most important requirements in quality life is healthy nutrition. Malnutrition causes many pathological and

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physiological causes in the body. Healthy nutrition is very important to protect from diseases. Besides physical activity, abstaining from alcohol consumption and smoking are important tips for healthy and quality living. Antioxidant nutrition is especially important to get rid of the effects of free radicals [5].

Acetylcholinesterase (AChE, E.C.3.1.1.7), which is involved in nerve transmission by hydrolyzing acetylcholine, is an enzyme in the family of cholinesterases. Acetylcholinesterase, which is responsible for hydrolyzing choline esters, has been determined in high concentration in brain, nerve, and red blood cells. The decline in AChE activity causes nervous system disorders and even death [6]. Acetylcholine (ACh) is an important substrate for the enzyme acetylcholinesterase [7]. The sudden drop in acetylcholine level is fatal. Gradual decline in this level leads to Alzheimer's disease (AD) [6]. The human brain contains approximately 100 billion neurons. AD is a neurodegenerative disease caused by damage or death of these cells [8]. Butyrylcholinesterase (BChE) is commonly found in tissues such as the lung, brain, heart, liver, muscle, kidney, and body fluids such as serum, sweat, and cerebrospinal fluid. BChE is an important enzyme in the origin of the liver. It is produced in the liver and can hydrolyze or activate a large number of different compounds. BChE has been used as a marker of liver function in clinical trials. AD is a neurodegenerative disorder with symptoms such as impaired cognitive abilities, reduced memory, and personality changes [9].

The association of AD with acetylcholine is well known. To ensure that ACh remains in the synaptic range is one of the methods used in the treatment of AD. For this purpose, cholinesterase enzyme inhibitors are used [10]. BChE is structurally similar to AChE. On the other hand, unlike AChE, it has various physiological tasks. Since BChE is found in serum and is expressed in the liver, the level of inflammation in the tissues, especially serum, varies in many pathological conditions such as the presence of inflammation, nutritional status, tumor and neurophysiological disorders [11]. Diabetes mellitus (DM) is one of the most important health problems. It is caused by insufficiency, lack or ineffectiveness of insulin hormone produced from beta cells of pancreas. When diabetes mellitus occurs, glucose molecules cannot be metabolized. Consequently, glucose increases in the body, and insulin hormone cannot be secreted regularly against increased glucose. α-Glucosidase enzyme is found on the brush surface of the small intestine. It is responsible for breaking down complex carbohydrates. The inhibitors of this enzyme help prevent hyperglycemia indirectly [6].

In pine forests, scots pine species are more common than other species. In Turkey, Northern Anatolia, East Anatolia, including Western Anatolia and Central Anatolia were examined under four different groups. Species grow predominantly in poorer, sandy soils, rocky tops, peat bogs, or near forest borders [12]. Scots Pine is a tree with a slender, cylindrical body with pointed hills and thin branches or fuller hulled, slender and thick branches, depending on the place of growth. Scots pine develops a narrowing hill by forming branches shortening towards the tip in early ages. The aged bodies of Scotch pine are gray-brown, thick and cracked. Scots pine needs little heat and water. It can grow in many different climates because it is frost resistant. Therefore, it can grow both at sea level and in high altitude regions. It is usually spread in mountainous regions but also in high ovals and narrow valley bases. Scots Pine is the tree of light sandy soil [13]. Scots pine grows in soils with low mineral content, and less moisture. Scots Pine wood contains 41.9% cellulose, 12.8% hexose, 8.7% pentosane, 29.5% lignin, 3.2% fat and 1.3% ash [27]. Scots Pine wood has a matte color. The resin is fragrant when it is green. It has a decorative appearance. This wood is as hard as possible and medium weight [14, 15]. Recently, the examination of the content of natural products has increased and this has opened the way for alternative medicine. Some parts of pine species including bark, needles, resin and cones are used as anti-inflammatory, antioxidant or antiseptic. For the reproduction of conifers, the presence and number of cones is also very important. As they protect and mature the seeds during development, the oaks opens and the seeds spread over a wide area [16]. It was well known that scots pine species had a common usage in folk medicine. The paste obtained by crushing scots pine seeds with honey is used as a strengthener. The scots pine seeds are pressed in the industry to obtain light yellow, odorless and delicious oil in Anatolian kitchen and confectionery. This oil is also used in the manufacture of soap, cosmetics and varnish [17]. Also, it was reported that some product obtained from Scots pine had tropical counter irritants fort the treatment of rheumatic disorders and muscle pain and as anti-aging cosmetics [18].

Foods of plant origin provide not only important antioxidant vitamins to life metabolism, but also natural compounds with antioxidant effect [11]. Recent studies have shown that compounds with antioxidant activity are highly effective in preventing many degenerative diseases such as cancer,

cardiovascular diseases, neurological diseases, and cataracts, caused by oxidative stress [19]. The antioxidant properties, and inhibition effects on AChE, BChE and α-glucosidase enzymes of *P. sylvestris* cones collected from Sarikamis (Kars), Gumushane, and Erzurum regions were examined in this study. The results obtained will be effective and guiding for food, cosmetic, alternative medicine, and pharmaceutical sectors. The inhibition of AChE, BChE and α-glucosidase enzymes and high antioxidant capacity will prevent these cones from health problems such as diabetes, AD, asthma, cancer, organophosphate intoxication, cocaine poisoning, rheumatoid arthritis, anesthetic apnea, cardiovascular diseases and small cell lung cancer. Phenolic contents of *P. sylvestris* shells have been studied in the literature using different methods [20]. Antioxidant activities, phenolic contents and enzyme inhibition of cones of this species were not studied. Hence, *P. sylvestris* cones, which are a natural product, have been studied and presented to the scientific community.

The main goal of this study is to determine the antioxidant activity of ethanol extracts of Scots pine (*Pinus sylvestris*) cones collected from different parts of Eastern Anatolia using by distinct bioanalytical methods including the cupric ($Cu^{2+}$) and ferric ($Fe^{3+}$) ions reducing abilities, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities. And to demonstrate the inhibitory abilities of the extract against the acetylcholinesterase, butyrylcholinesterase and α-glucosidase enzymes, which are linked to global and common health diseases and the secondary metabolite profile of extract of species were determined and structure activity relationship evaluated herein.

## 2. Materials and Methods

### *2.1. Plant Material and Extraction*

*P. sylvestris* cones were collected in June from Erzurum, Sarikamis (Kars) and Gumushane provinces of Turkey. A voucher specimen of Gumushane (ATA 9878), Erzurum (ATA 9879) and Sarikamis (ATA 9880) *P. sylvestris* cones has been deposited at Atatürk University Faculty of Science Herbarium. 100 g of dried cones of each sample shredded with a blender and 150 mL of ethyl alcohol were added and macerated 24 hours. Then the mixture was filtered through filter paper and the remaining pulp was further extracted with 100 mL ethyl alcohol under the same conditions and filtered again. Then the extracts were combined, and ethanol was removed in the rotary evaporator at 35°C under vacuum. The extracts were placed in plastic bottles, and then stored at -20°C until further experiments.

### *2.2. Antioxidant Capacity Assays*

$Fe^{3+}$ reduction capacity and $Cu^{2+}$ reduction capacity (Cuprac method) assays were performed according to the method of Oyaizu et al. [21] and Apak et al., respectively [22]. DPPH free radical scavenging activity was performed according to Blois method as described in previous studies [23] and ABTS radical scavenging activity was determined according to the method of Re et al. [24]. Detailed informations of applied methods are given in supporting information.

### *2.3. Determination of Total Phenolic and Flavonoid Contents*

Total phenolic contents in *P. sylvestris* cones were determined by using Folin-Ciocalteu regent [2] and The total flavonoid content of cone extracts was determined by using the method of literature and quercetin used as a standard in this method [9]. Detailed information of applied methods are given in supporting information.

### *2.4. Enzyme Activity Assays*

AChE, and BChE inhibition activities were carried out according to the Ellman's method [25] and α-glucosidase enzyme (E.C.3.2.1.20) inhibition patterns were determined via the well known procedure and *p*-NPG was used as a substrate [6]. Detailed information of applied methods are given in supporting information.

*2.5. Statistical Analysis*

Each experiment was performed three times. The obtained data were recorded as mean ± standard deviation and analyzed with SPSS (Windows 2000, version 11.5 for SPSS Inc., Chicago, IL). Variance ANOVA including one-way analysis was realized. Significant differences between the averages were determined by Duncan's Multiple Range tests.

*2.6. Chromatography conditions*

Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) measurements were performed with a Thermo Orbitrap Q-Exactive instrument in ESI Source and equipped with a Fortis C18 column (150 mm x 2.1, 3 μm particle size). The mobile phase was composed of water (A, 0.1% formic acid) and methanol (B, 0.1% formic acid), the gradient programme of which was 0-1.00 min 40% A and 60% B, 1.01-05.00 min 100% B and finally 60-10 min 40% A and 60% B. The flow rate of the mobile phase was 0.30 mL/min, and the column temperature was set to 22°C. The injection volume was 2 μL. The best mobile phase solution was determined to be a gradient of acidified methanol and water system as reported above. Such a mobile phase was determined to be satisfactory for the ionization abundance and separation of the compounds. The good ionization of small and relatively polar antioxidants was obtained by the ESI source. The ions between *m/z* 85-1200 were scanned in high-resolution mode of instrument. Identification of compounds was done by comparison of retention time of standard compounds (in the range of purity 95% - 99%) and HRMS data of Bezmialem Vakif University, Drug Application and Research Center Library (İLMER). Freshly prepared curcumin (purity 97 %) used as an internal standard.

The working solutions are prepared as follow. 168.5 mg (Erzurum), 234.2 mg (Sarikamis) and 120.9 mg (Gumushane) of methanol extracts of *P. sylvestris* weighed and dissolved in 5 mL of with a 2.5 mL of mobile phase (Mobil Phase A: B; 50:50). Then, from 100 mg/L internal standard solution is added to the final concentration of 3 ppm and volume was completed to 5 mL with mobile phase. Then, the solution was filtered through a 0.45 μ filter and 2 μL was added to the instrument [26].

## 3. Results and Discussion

*P. sylvestris* is a species of the genus *Pinus* of the *Pinaceae* family from the Gymnospermae class. *P. sylvestris* has the widest geographic distribution of species present in the pine species. *P. sylvestris* constitutes 5.5% of the total forest area in Turkey. In the area covered by coniferous plants in Turkey, *P. sylvestris* ranks 3$^{rd}$ after *P. nigra* and *P. brutia* [27]. It is aimed to produce male and female flowers, which are the main objective in seed gardens. Accordingly, seeds produced with cone and seed yield should be of good quality. It is very important to determine differences between cones [28]. The cones may differ in their different populations. Seed retention depends largely on the genetic characteristics of the tree species and climate variability. Light seeded trees hold usually seeds earlier than their heavy seeds. Light trees also hold seeds at earlier ages than shade trees. *P. sylvestris* indicates that the seed rate is low and sparse at high altitudes [29]. Forest trees begin to hold cones at certain ages. Retention of cones varies depending on the type of aging, genetic structure, characteristics of the growing environment and climate factors. Pile of cones and cones holds abundantly in poor and unfavorable soils for fear of termination of trees [30].

*3.1. Determination of Secondary Metabolites*

It has been determined that the phenolic components of the secondary metabolites are identified as anti-inflammatory, antitumor, antiviral, antiallergic, antimicrobial, antimutagenic, antioxidant, and anticancer agent [26]. Phenolic compounds effectively prevent oxidation in nutrient systems and serve as a protective factor against oxidative damage in the human body [9].

Thus, it is very important to understand the mechanisms of action of phenolic and flavonoid substances which have important effects on human health and to investigate the ways in which they can

be used [1]. Total phenolics and total flavonoid contents of the ethanol extracts of Sarıkamis, Gumushane and Erzurum *P. sylvestris* cones were determined herein (Table 1).

**Table 1.** Total flavonoids content as quercetin equivalent (QE/mg of extract) and total phenolic content as gallic acid equivalent (GAE/mg of extract) in *P. sylvestris* cones

| Antioxidants | Total Phenolic (µg GAE/mg) | Total Flavonoid (µg QE/mg) |
|---|---|---|
| Sarikamis | 99.09 | 10.22 |
| Erzurum | 123.64 | 12.22 |
| Gumushane | 131.82 | 14.44 |

In addition to those data, determination of phenolic acid contents of *P. sylvestris* cones were analyzed by using LC-HRMS. Table 2 shows the information on the concentration of identified secondary metabolites in ethanol extracts and the sample chromatograms are given for ethanol extract of *P. sylvestris* in Figure 1.

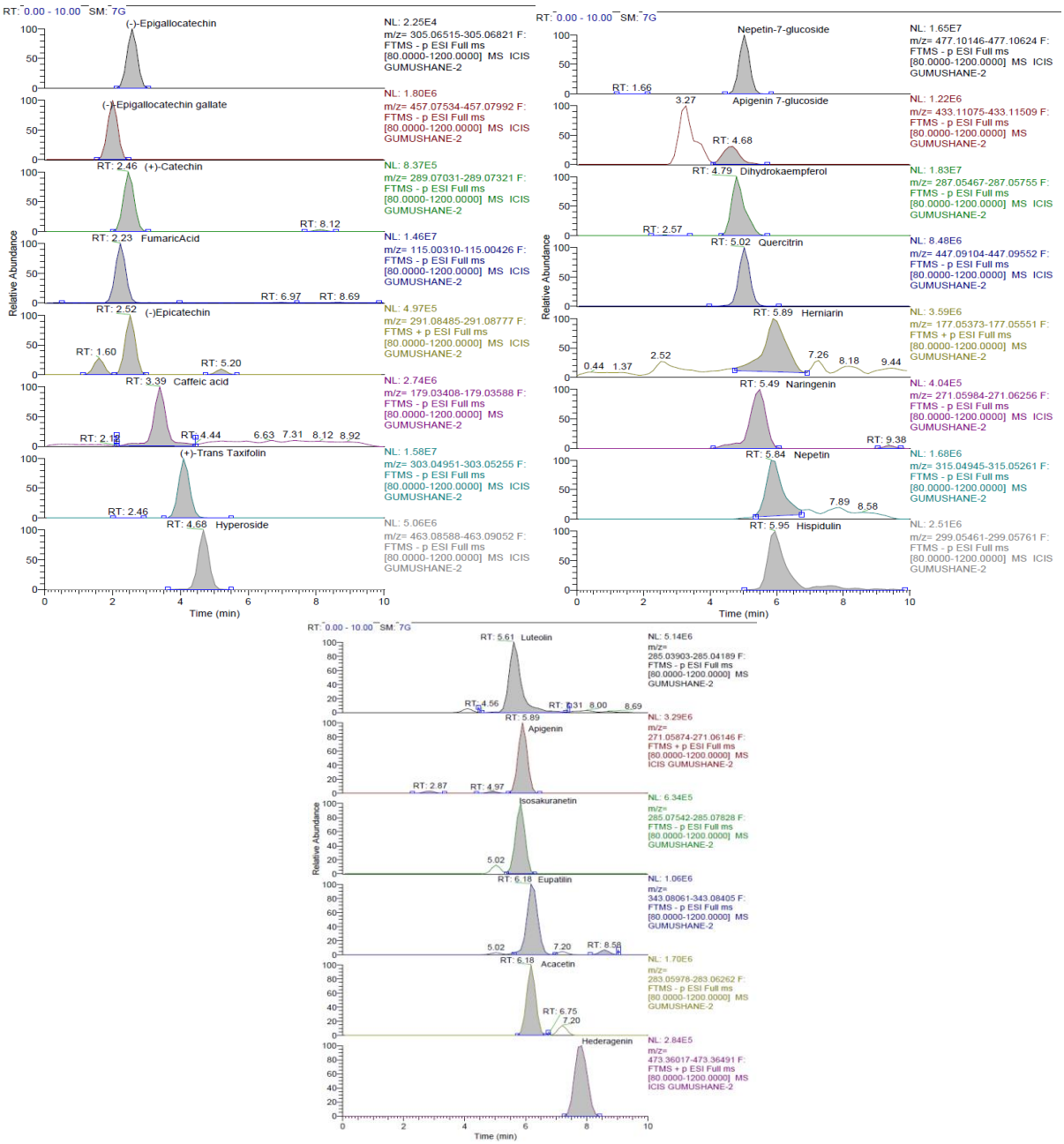


**Figure 1.** LC-HRMS chromatogram of ethanol extract of *P. sylvestris* cone (Gumushane)

The mass parameters and linear regression equations of were reported for the compounds by LC-HRMS (See Table S1). Measurement uncertainty values are given together with the results in Table 1 % U (k = 2, 95% confidence interval). Measurement uncertainty is determined in accordance with GUM and EA-4/02 documents.

The results showed that the ethanol extract of Gumushane *P. sylvestris* cone (131.82 µg GAE/mg) contained more total phenolic compounds and flavonoids (14.44 µg QE/mg) than the others (Table 2). Those data shows the strong correlation of antioxidant capacity and phenolics contents of the extracts as described before [26]. In order to identify the phenolic contents of the extracts, secondary metabolite composition of them were analyzed by LC-HRMS (see Table S1 and Table 3).

**Table 2.** Compounds determined in *P. sylvestris* cone extracts and their amounts (g extract)

| Compounds | Erzurum | Sarikamis | Gumushane | U % (k=2) |
|---|---|---|---|---|
| (-) Epigallocatechin | 0.01 | 0.01 | 0.03 | 3.11 |
| (-) Epigallocatechin gallate | 0.11 | 0.05 | 0.45 | 2.73 |
| (+) Catechin | 0.62 | 0.50 | 0.05 | 1.84 |
| Fumaric Acid | 2.25 | 1.13 | 5.51 | 3.15 |
| (-) Epicatechin | 1.32 | 1.12 | 0.08 | 3.62 |
| Verbascoside | 0.01 | <LOD | <LOD | 3.59 |
| Caffeic acid | 0.19 | 0.76 | 0.13 | 2.41 |
| Luteolin-7-rutinoside | 0.01 | <LOD | <LOD | 1.43 |
| Hesperidin | <LOD | 0.02 | <LOD | 2.82 |
| (+) *trans* Taxifolin | 2.61 | 1.01 | 1.12 | 2.97 |
| Hyperoside | 3.08 | 0.48 | 1.75 | 3.01 |
| Rosmarinic acid | <LOD | <LOD | <LOD | 4.38 |
| Nepetin-7-O-glucoside | 1.56 | 0.56 | 1.64 | 4.39 |
| Apigenin 7-O-glucoside | <LOD | 0.26 | 0.33 | 3.13 |
| Dihydrokaempferol | 0.28 | 0.22 | 1.06 | 3.80 |
| Quercitrin | 0.61 | 0.29 | 0.61 | 4.78 |
| Myricetin | 0.02 | 0.01 | <LOD | 1.71 |
| Herniarin | 0.01 | <LOD | 0.01 | 0.94 |
| Naringenin | 0.02 | 0.04 | 0.04 | 4.15 |
| Nepetin | 0.01 | <LOD | 0.02 | 3.21 |
| Rhamnocitrin | <LOD | 0.01 | <LOD | 2.76 |
| Hispidulin | 0.19 | 0.06 | 0.40 | 1.73 |
| Kaempferol | 0.03 | 0.02 | <LOD | 0.91 |
| Luteolin | 0.01 | 0.01 | 0.03 | 1.91 |
| Apigenin | 0.02 | 0.04 | 0.04 | 2.72 |
| Isosakuranetin | 0.26 | 0.09 | 0.20 | 1.21 |
| Eupatilin | 0.16 | 0.08 | 0.31 | 1.38 |
| Chrysin | 0.01 | <LOD | <LOD | 1.19 |
| Acacetin | 0.10 | 0.02 | 0.08 | 1.50 |
| Quillaic acid | 0.09 | 0.05 | <LOD | 3.01 |
| Hederagenin | 0.17 | <LOD | 0.41 | 9.23 |

The major compounds are determined as hyperoside, *trans* taxifolin, nepetin-7-o-glucoside, apigenin 7-O-glucoside, dihydrokaemferol, hispudilin, eupatulin and hederaganin. In addition to those the highest content of fumaric acid determined in that extract. Thus we clearly conclude that the strong antioxidant capacity of extract of *P. sylvestris* from Gumushane comes from those secondary metabolites (Table 2).

*3.1. Antioxidant Capacity*

The reduction capacity of a compound can be measured by different methods. One of the most commonly used methods for this purpose can be measured by reducing $[Fe(CN]_6]^{3+}$ to $[Fe(CN)_6]^{2+}$[27]. Comparing the reduction forces of ferric ions ($Fe^{3+}$) of the standard antioxidants and the *P. sylvestris* cones at 20 µg/mL concentration: BHA ($r^2$: 0.9539) > Trolox ($r^2$: 0.9138) > α-Tocopherol ($r^2$: 0.9938) > BHT ($r^2$: 0.9685) > Gumushane ($r^2$: 0.9912) > Erzurum ($r^2$: 0.9909) > Sarikamis ($r^2$: 0.9937). In the present study, the capacity to reduce the ferric ions ($Fe^{3+}$) to the ferrous ions ($Fe^{2+}$) as well as the reduction of cupric ions ($Cu^{2+}$) to the cuprous ions ($Cu^{+}$) was also studied. Cuprac method has gained widespread use in recent years [31]. This method is a low cost, fast, stable and convenient method. The activity of reducing cupric ions ($Cu^{2+}$) of the *P. sylvestris* cones and standard antioxidants at 20 µg/mL concentration are compared with each other: BHA ($r^2$: 0.8886) > BHT ($r^2$: 0.9675) > Trolox ($r^2$: 0.9998) > Gumushane ($r^2$: 0.9999) > Erzurum ($r^2$: 0.9806) > α-Tocopherol ($r^2$: 0.9786) > Sarikamis ($r^2$: 0.9760).

As shown above, antioxidant and antiradical studies were compared with standard antioxidants such as α-Tocopherol, BHT, BHA, and Trolox. In most antioxidant models, *P. sylvestris* cones of equal concentrations and activities of standard antioxidants showed that Gumushane *P. sylvestris* cones had higher activity. Reduction capacity is one of the most important factors in the antioxidant activity of a compound. The reducing power of a compound is related to antioxidant activity [31].

The methods based on the use of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals are the spectrophotometric methods commonly used to determine the antioxidant capacity of pure substances, food, beverages and vegetable extracts [8]. The DPPH radical is a stable long-life nitrogen-free radical. It is one of the most commonly used methods to determine the radical scavenging activity of antioxidants [19].

When the $IC_{50}$ values of DPPH radical scavenging activity of *P. sylvestris* cones and the standard antioxidants were compared, it was observed that the Gumushane *P. sylvestris* cone (14.75 µg/mL) had a higher $IC_{50}$ value than the other cones (Table 1). Trolox (7.62 µg/mL) has a relatively high $IC_{50}$ value. These values have been observed to be quite high when compared with some pure antioxidants isolated from purified vegetal sources. For example, DPPH radical scavenging activities of both alcohol and water extracts of flaxseed were calculated as 53.30 and 49.50 µg/mL, respectively [4]. $IC_{50}$ values of *P. sylvestris* cones were calculated between 14.75-30.13 µg/mL (Table 3). When the results were examined, it was observed that cones have higher antioxidant activity than curcumin (34.9 µg/mL) determined in the literature.

**Table 3.** $IC_{50}$ (µg/mL) values of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals scavenging activities of *P. sylvestris* cones and standard antioxidants

| **Antioxidants** | **DPPH· scavenging** | **$ABTS^{\bullet+}$ scavenging** |
|---|---|---|
| Sarikamis | 30.13 | 14.63 |
| Erzurum | 21.00 | 12.82 |
| Gumushane | 14.75 | 12.56 |
| BHA | 9.90 | 11.73 |
| BHT | 12.38 | 11.77 |
| α-Tocopherol | 12.38 | 12.11 |

The radical scavenging activities of antioxidant compounds are very important in eliminating the damage caused by free radicals to the organism in the biological systems, food and pharmaceutical industry. In these systems, free radicals occur and accelerate lipid peroxidation, thus reducing the quality of products [32]. In the study, Table 2 shows that the *P. sylvestris* cones used have been able to scavenge ABTS radicals quite strongly. In the determination of ABTS radicals, $IC_{50}$ values of ethanol extracts of *P. sylvestris* cones were found to be 12.56-14.63 µg/mL. These values were found to be parallel to the standard antioxidants as shown in Table 3.

*3.2. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Activity*

Acetylcholinesterase (AChE) is an enzyme that hydrolyses the acetylcholine compound involved in the communication between synapses in the nervous system. It is usually a member of the family of carboxylesterase enzymes as found in the synapses of the muscle and brain nerves [36]. Butyrylcholinesterase (BChE) synthesizes in liver and releases into circulation. The classic role of BChE is the hydrolysis of succinylcholine, which is used as a local anesthetic. Succinylcholine is used as a short-term muscle relaxant prior to general anesthesia in surgery. BChE is the only enzyme that can break down succinylcholine in the organism [19]. The substrate selectivity of cholinesterases is different. AChE can rapidly hydrolyze a natural neurotransmitter, acetylcholine, compared to other large acyl-tailed choline esters, while BChE can rapidly hydrolyze synthetic substrates, mainly butyrylcholine [11].

When this disease is treated using by cholinesterase inhibitors, the acetylcholine becomes normal levels. Consequently, cognitive and behavioral disorders can be treated to a certain extent. In our study, the inhibition levels of cones, a natural product, on these two enzymes were determined. $IC_{50}$ values are as given in Table 4. The calculated values were determined to be quite effective compared to natural products. Especially, the Gumushane *P. sylvestris* cone was found to be the most effective inhibition (AChE and BChE) of cones. According to the results, it can be used in the treatment of AD.

**Table 4.** $IC_{50}$ values of the enzyme inhibition results

| Region | AChE (mg/mL) | BChE (mg/mL) | α-Glucosidase (mg/mL) |
|---|---|---|---|
| Sarikamis | 7.96 | 2.72 | 43.31 |
| Erzurum | 9.91 | 3.48 | 40.76 |
| Gumushane | 6.41 | 2.22 | 26.65 |
| Tacrin | 0.97 | 1.66 | - |
| Acarbose | - | - | 0.015 |

*3.3. α-Glucosidase Activity*

Oxidative stress is one of the important markers of Type-2 diabetes [19]. The antioxidant molecules used to minimize the effects of oxidative stress can also be used as an inhibitor of α-glucosidase enzyme [6]. Inhibition effects of cones from different regions in Eastern Anatolia on α-glucosidase enzyme were investigated and the results are showed in Table 3. Sarikamis and Erzurum *P. sylvestris* cones showed close inhibition while Gumushane *P. sylvestris* cone (26.65 mg/mL) showed more effective inhibition. $IC_{50}$ value of acarbose, which used as the α-glucosidase inhibitor, was found to be 0.015 mg/mL. It is thought that cones obtained from natural product will guide oral diabetic drug groups according to these values [32].

As a conclusion, *P. sylvestris* cones in 3 from different province of Turkey with different terrestrial climates were compared herein. Both enzyme inhibition values and antioxidant capacities of Gumushane *P. sylvestris* cone were found to be more effective than other cones. The reason is thought to be due to the fact that it's high phenolic content. A similar study is reported on the radical scavenging effects, total flavonoid and phenolic contents of *P. sylvestris* of water extracts of whole bark and outer bark grown in the coastal and continental regions of Kaliningrad region (Russia) were reported with a small amounts of tannin contents and flavonoid content are reported as 2.1-5.2 mg/g [33]. According to those results, I clearly state that the cone extracts of *P. sylvestris* may be a source of natural antioxidants and anticholinesterase products.

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## Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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